HYBRIDOMA
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Monoclonal Antibodies

1B2.1E12 anti-TNP

ANTIGEN USED FOR IMMUNIZATION

OVA-TNP conjugate: Type VI ovalbumin (OVA) and 2,4,6-trinitrobenzenesulfonic acid (TNBS), both from Sigma Chemical Co. (St. Louis, MO), were conjugated as described.[1]

METHOD OF IMMUNIZATION

BALB/c mice were immunized intraperitoneally with 100 μg of TNP-OVA conjugate and complete Freund's adjuvant (1:1, v/v). On Days 20 and 30, the animals were boosted with the same dose of antigen diluted in 0.15 M saline. Three days later, immunized mice were sacrificed in a CO_2 chamber, and their spleen cells were aseptically collected for fusion experiment.

PARENTAL CELL LINE USED FOR FUSION

SP2/0 Ag-14

SELECTION AND CLONING PROCEDURES

Spleen cells of three immunized mice were pooled and then fused with SP2/0 Ag-14 myeloma cells (4:1 ratio) by mixing them with polyethylene glycol:dimethylsulfoxide (PEG: DMSO; 50%:10%) as described.[2] After fusion, the cells were suspended in HAT medium, dispensed into each well of two 24-well flat bottomed tissue culture plates containing macrophage feeder layers, and then cultured at 37°C in a humid atmosphere of 5% CO_2 in air. The hybridoma cells producing anti-TNP antibodies, as detected by ELISA using TNP-BSA, were cloned by limiting dilution in 96-well microplates (Corning) containing macrophage feeder layers.

HEAVY AND LIGHT CHAINS OF IMMUNOGLOBULIN

$IgG_{1\kappa}$

SPECIFICITY

The supernatants and ascites obtained from the clone 1B2.1E12 were assayed for their reactivity with TNP-OVA, TNP-BSA, OVA, and BSA. These materials gave positive reactions only when TNP-BSA was used as antigen in ELISA tests.

SPECIFIC ANTIGEN IDENTIFIED

TNP

AVAILABILITY

Tissue culture supernatant	Yes ✓	No
Ascitic fluid	Yes ✓	No
Hybridoma cells	Yes ✓	No

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